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Substrate-Borne Vibrational Communication in Veiled Chameleons (*Chamaeleo calyptratus*) During Courtship, Breeding, and Territoriality

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SUBSTRATE-BORNE VIBRATIONAL COMMUNICATION IN VEILED CHAMELEONS (*CHAMAELEO CALYPTRATUS*) DURING COURTSHIP, BREEDING, AND TERRITORIALITY

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I dedicate this thesis to my mother, Kimberly Tabb, who bestowed upon me the gift of perserverance, allowing me to achieve goals regardless of how life plays out.

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Lauren Kappel May 2020 46 pages

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Substrate-borne vibrations, or biotremors, are utilized by vertebrates found in unique environments because biotremors are an effective way to transmit signals through dense media. Previous studies have shown that veiled chameleons (*Chamaeleo calyptratus*) are able to produce biotremors via specialized neck muscles. I hypothesized that during courtship and/or breeding, the veiled chameleon, a tree-dwelling species, would communicate with biotremors through branches. Additionally, I hypothesized that female call characteristics would differ between reproductive condition (i.e., receptive and non-receptive), while male call characteristics would differ between behavioral contexts (i.e., territorial vs. courtship). Chameleons were paired (one male, one female) and placed on a wooden dowel to create an optimal setting for reproductive behavior, and the biotremors produced by each chameleon were recorded with accelerometers and characterized by the type of call (hoot or rumble), duration (s), and dominant frequency (Hz). Linear mixed-effect models and ANOVAs were used to analyze the effect that variables such as sex, receptivity of the female, snout-vent length (SVL), and behavioral context had on the biotremors being produced. Both receptivity of females and SVL had an effect on female hoot and rumble frequencies, while receptivity of females had an effect on the duration of female rumbles. Additionally, behavioral context affected male

hoot frequency. These results reflect how females could be able to advertise receptivity utlilizing biotremors. While males could potentially communicate their presence to females or competing males within range.

INTRODUCTION

Whether detecting prey, avoiding predators, or identifying and attracting potential mates, animals utilize multiple modes of communication to survive. In a functional communication system, a signaler conveys information (signal) to a receiver, resulting in a physiological or behavioral change in the recipient (Otte, 1974; Dawkins and Krebs, 1978; Markl, 1983; Endler, 1993; Bradbury and Vehrencamp, 2011). Otte (1974) defines signals as "behavioral, physiological, or morphological characteristics fashioned or maintained by natural selection because they convey information to other organisms". The most common types of signals fall under the categories of auditory, visual, and chemical communication. Some auditory communication functions as a warning to alert conspecifics about predators, like the 'chirt' of the Carolina wren (*Thryothorus ludovicianus*) (Morton and Shalter, 1977). The horned frog (*Ceratophrys ornate*) curls/twitches its middle three toes on one or both hind feet to mimic a moving worm when encountering potential prey; this type of deceitful visual communication is termed 'pedal luring' (Hödl and Amézquita, 2001). Communication also plays a role in mating contexts, such as the ether extracts emitted by virgin queen honeybees (*Apis mellifera* L.) in order to attract potential drones during mating flights (Gary, 1962).

Each form of communication consists of costs and benefits to the species that utilize them (Endler, 1993; Seyfarth and Cheney, 2003; Römer et al., 2010). For example, male túngara frogs (*Physalemus pustulosus*) 'whine' in order to attract females and deter competing males (Ryan, 1980; Seyfarth and Cheney, 2003). However, when the male frogs add a 'chuck' to the whine, it not only makes them more attractive to the females, but also makes it easier for predatory bats to locate them (Rand and Ryan, 1981; Seyfarth

and Cheney, 2003). Similarly, the male sharp-tailed grouse (*Tympanuchus phasianellus*) must compete with other males in visual displays to court females, but these same displays also attract predatory eagles (Bradbury and Vehrencamp, 2011). In both examples, there is both sexual selective and natural selective pressures working against one another as a cost/benefit trade-off within these communication systems.

One form of communication that has received very little attention within vertebrates, despite being relatively common in other groups like insects, is vibrational communication, or biotremors (for exceptions, see Hartline, 1971; Barnett et al., 1999; Christensen et al., 2012; Laslie, 2018; Tegge, 2018). Hill and Wessel (2016) define biotremology as the study of communication by substrate-borne vibrations, which are detected by specialized perception organs that measure the oscillations at a boundary between media. The mechanisms necessary to receive and translate information conveyed through substrate-borne vibrations are widely present in vertebrates and arthropods (Hill, 2009). It has been estimated that at least 150,000 species of insects use vibrational signaling as their exclusive communication method (Virant-Doberlet and Cokl, 2004; Cocroft and Rodríguez, 2005; Cocroft et al., 2006). One benefit of utilizing vibrations is that the signal can only be detected by individuals that are on or in close proximity to the substrate along which the signal was sent (Barnett et al., 1999; Cocroft and Rodríguez, 2005), which makes eavesdropping less likely. However, there are still ways for an unintended receiver to intercept a message. For example, the egg parasitoid (*Telenomus podisi*) is able to detect and orient themselves towards vibrations produced by female brown stink bugs (*Euschistus heros*) during sexual communication (Laumann et al., 2007). Most vibrational communication studies are based on insects and other arthropods,

yet there are some studies demonstrating vibrational communication in reptiles (Hartline, 1971; Barnett et al., 1999; Christensen et al., 2012).

Chameleons are a monophyletic group of reptiles originating from East Africa (Nečas, 1999; Tolley and Herrel, 2013). Of the chameleon species described, 55% can be found in Africa, 44% in Madagascar, and the remaining species can be found in Asia and Europe (Tollet and Herrel, 2013). Chameleons inhabit a wide range of environments, from open-canopy habitats such as savanna, grassland, and woodland, to closed-canopy habitats, or forests (Tolley and Herrel, 2013). While the Chamaeleonidae display a vast amount of diversity across species, there are many unique features all chameleons possess that set them apart from other lizards. These features include forcep-like feet, a prehensile tail, independently moving, turreted eyes, and a ballistic tongue capable of grasping prey (Mates, 1978; Nečas, 1999; Tolley and Herrel, 2013; Huskey, 2017). Chameleons primarily communicate through the use of visual cues due to their skin color-changing ability (Tolley and Herrel, 2013). Specialized skin cells called 'chromatophores' are the mechanism by which they can send these visual signals. There are four broad categories of color change responses in chameleons: temperature, light, physiological state, and sensory input (Tolley and Herrel, 2013). Color changing due to physiological state is perhaps the most relevant to this project, as female chameleons are able to communicate their reproductive status via color patterns (Kelso and Verrel, 2002; Tolley and Herrel, 2013).

Visual communication is a critical function of courtship and breeding in the veiled chameleon, *Chamaeleo calyptratus*. When approached by a male, a receptive female will maintain a light green background skin color, while exhibiting faint blue spots along her

dorsal surface (Annis, 1995; Kelso and Verrel, 2002). Non-receptive females will typically darken their background coloration to black or brown when approached by a male, highlighting orange and blue spots on their dorsal surface (Annis, 1995). In addition to coloration, female veiled chameleons communicate their sexual status via movement behaviors. For example, a non-receptive female will demonstrate aggressive behaviors (e.g., rocking back and forth, gaping, or hissing), while receptive females will typically remain stationary when approached by a potential mate (Kelso and Verrel, 2002; Pimm et al., 2014). Upon approaching females, males will initiate courtship with a 'lateral display' in which they will display bright colors and compress their body laterally in front of the female (Kelso and Verrel, 2002). Then, depending on female reproductive status, the male will approach the female from the side or behind and exhibit more copulatory behaviors, such as a head roll, chin rub, head butt, or a vibration, which are dependent on reproductive status as well (see Kelso and Verrel, 2002).

In addition to visual cues, veiled chameleons have also been reported to communicate with biotremors during courtship interactions (Barnett et al., 1999). Barnett et al. (1999) found that male veiled chameleons did not produce vibrations when alone but did produce vibrations when introduced to a female. The mean frequencies of these vibrations ranged from 50-149 Hz (Barnett et al., 1999). Tegge (2018) determined that during the production of biotremors, four hyoid muscles were being activated: *Mm. mandibulohyoideus, M. levator scapulae, M. sternohyoideus superficialis,* and *M. sternohyoideus profundus*. During the contraction of these muscles, the gular pouch, located between these muscles, is hypothesized to already be inflated and act as a 'resonator' to amplify the vibrations produced (Tegge, 2018; Huskey et al., 2020). *C.*

calyptratus have been observed to produce biotremors for both intra- and interspecific interactions in numerous behavioral contexts including alarm calls, territorial aggression, and mating (Barnett et al., 1999; Laslie, 2018; Tegge, 2018).

The purpose of this study is to investigate the vibratory signals used by veiled chameleons while communicating during courtship and breeding. The specific questions addressed in this study are: (1) Is there reciprocal calling between male and female veiled chameleons during courtship and breeding? (2) If so, do the call characteristics differ between non-receptive and receptive females? And (3) Do the male call characteristics differ between behavioral contexts (e.g., territorial vs. courtship)? Determining how call characteristics vary in different situations (i.e., reproductive status or behavioral context) will aid in understanding this novel form of communication and its function in *C. calyptratus*.

MATERIALS AND METHODS

Chameleon care and husbandry

Ten adult *Chamaeleo calyptratus* (five females, five males) were used in this study (*Table 1*). The chameleons were commercially sourced from LLL Reptile, Inc. and Backwater Reptiles, Inc. and maintained in accordance with specifications set by Nečas (2004).

Each chameleon was housed individually in a wire mesh enclosure. Larger males (snout-vent length greater than 175 mm) were kept in 61 × 61 × 122 cm enclosures, while all other individuals were kept in 46 × 46 × 92 cm enclosures. In order to prevent territorial aggression and reduce stress, the cages were separated adjacently with opaque barriers. Enclosures were equipped with foliage and 60-watt ceramic heat and UVB lamps powered on a 12:12 light-dark cycle. Continuous drip systems were placed on top of each enclosure and the chameleons were misted daily to maintain hydration. Chameleons were fed 5-7 gut-loaded crickets (*Acheta domesticus*) every other day. In order to maintain an optimal environment, the temperature in the room was kept at 24°C and the humidity at 50% or higher using a Levoit (6L) humidifier.

Experimental and video analysis procedures

All protocols were compliant with guidelines established by the Institutional Animal Care and Use Committee (IACUC) of Western Kentucky University (Animal Welfare Assurance #A3448-01).

For each trial, one male and one female chameleon were placed onto a wooden dowel (77 cm length, 1.0 cm diameter) inside an experimental chamber composed of a wooden box (47 × 94 × 54 cm) with a Plexiglas front panel and a closed top (*Figure 1*).

Vibrations were recorded using PCB Piezotronics, Inc. accelerometers (Model 394C06), mounted on each individuals' casque with PCB Piezotronics, Inc. petro wax (Model 080A24; see *Figure 2*). The vibrations produced by each chameleon were recorded using Video DAQ Acquisition and Analysis software designed using LabVIEW (created in LabVIEW by Dr. Doug Harper, WKU Physics Department).

Each trial was conducted in a sound dampening room (SE 2000 Sound Isolation Enclosure, WhisperRoom, Inc.). Recordings began once both the male and female chameleons were placed into the enclosure and accelerometers were attached to their casques.. Vibrations from both animals and video were recorded simultaneously in order to match visual behaviors with the calls being produced, and to later characterize the female in each trial as receptive or not, and to determine whether copulation occurred. Recordings were stopped either after copulation had occurred, or after approximately ten minutes if the female was non-receptive.

In order to determine the effects that behavioral context had on male call characteristics, male territorial calls recorded in a previous study (Laslie, 2018) were utilized for data analysis. Territorial trials differed, as there was a Plexiglas insert that separated the experimental chamber into two. The insert contained a hole, allowing the use of a single dowel. An opaque fabric barrier was placed in front of the Plexiglas insert prior to the placement of the two individuals. Each male was placed on either side of the chamber, with wired accelerometers attached to each of their casques. Five minutes after recording had begun, the opaque fabric barrier was removed so each individual was able to see the other. All calls were characterized the same as in this study. The calls produced

by the males, subsequent to the barrier removal, were used in the data analysis part of this study.

Data analysis

The LabVIEW Video DAQ Analysis Software was used to analyze video and vibration data simultaneously (*Figure 3*, *4*) as well as to export each recording to a *.wav* file in order to characterize each individual biotremor using Audacity software (version 2.3.2). Each *.wav* file was played-back twice, once for the male accelerometer recording and once for the female, in order to characterize each biotremor produced. The following was documented for each biotremor: onset time (to later determine reciprocal call latency), duration (s), dominant frequency (Hz), and the type of call produced (hoot or rumble). The dominant frequency was measured using the dominant peak in the power spectra that were calculated using a 1024 point Fast Fourier Transform (FFT) with a Hanning window. Hoots were easily distinguishable with a characteristic tonal "hoot"-like sound, while rumbles were less obvious with a lower frequency sound similar to a cat purring.

Statistical analysis

Linear mixed effect models were generated using the lme4 (Bates et al., 2015) and lmerTest (Kuznetsova et al., 2017) packages in R, in order to compare biotremor characteristics. Duration and dominant frequency were used as the response variables. Because hoots typically had a shorter duration and higher frequency than rumbles, separate models were used for each type of biotremor. The individual chameleon was set as a random effect, as multiple calls were produced by the same individuals. Snout-vent length (SVL) was also incorporated into these models as a covariate, to determine whether size had an effect on the variation in the response variables. Receptivity of the

female was used as a fixed effect to address the question of whether or not female call characteristics differ between non-receptive and receptive females.

The same process was employed to answer the question of male call characteristics differing with behavioral context. Male only data from this study was pooled with previously recorded data from eight male-male territorial trials (N=168) consisting of three individuals (Laslie, 2018). Linear models were created as mentioned above, with behavioral context set as the fixed effect rather than receptivity of the female. SVL was not used as a covariate for this dataset, as there were inconsistencies with the measurements from the previous trials.

Courtship trials were also analyzed to determine whether or not reciprocal calling occurred. Reciprocal calling was considered to occur when inter-call intervals between individuals took place within a 20-second period. This time frame was selected based on the observation of a clear unimodal distribution in delays between calls (*Figure 5*). That is, the majority of calls from both individuals either occurred within 20 seconds.

Linear mixed effect models were also used to determine if call rate (number of calls/trial) differed based on whether or not copulation had occurred in the trial. Copulation was considered to have occurred if the male mounted the female at any point during the trial. The linear models were created with duration and dominant frequency as response variables, copulation or no copulation as the fixed effect, and individuals as random effects.

RESULTS

A total of 1,052 calls were recorded from 37 male-female courtship trials. The two main types of calls were single hoots and rumbles (*Figure 6*), comprising 62.93% and 31.84% of calls, respectively. Mean duration (s) and mean dominant frequency (Hz) of each call type varied across individuals (*Table 2*) with some individuals (e.g., F2) producing higher frequency hoots than others (e.g., F5). Some individuals also produced longer calls than others (e.g., M5 produced longer rumbles than M1). Additionally, four different combinations of hoots and rumbles were recorded. All combination calls were continuous, with one call leading to another call. These combinations consisted of double hoots (0.57%), rumble-hoot-rumbles (0.57%), rumble-hoots (1.71%), and hoot-rumbles (2.38%). Because the combination calls only made up approximately 5% of total call density, only single hoots and rumbles were used for analysis.

Trials were categorized by receptivity of the female, with 22 consisting of receptive females and 15 with non-receptive females. Reciprocal calling between male and female *C. calyptratus*, which was defined as inter-call intervals under 20 seconds, occurred in 14 of the 37 trials (*Table 3*). The average latency between male and female calls was 6.92 s (SD=6.12). The percentage of reciprocal calls within these trials ranged from 1.22%-35.29%.

Of the 22 trials with receptive females, 15 consisted of copulation, or mounting. Barplots of the mean call rates (*Figure 7A, 7B*) indicated that there was a much larger amount of standard error when one of the individuals, F2, was included compared to when she was excluded from the data (*Figure 7C, 7D*). The linear mixed effect model for the male-female pooled dataset was repeated with the same dataset excluding F2. Call

rate did significantly differ with copulation ($F_{1,70.2}$=6.5, p=0.013) (*Table 4A, Table 5A*). Trials with copulation consisted of a higher call rate (mean=51.6) than trials without copulation occurring (mean=12.6). Conversely, in trials excluding F2, call rate did not differ significantly with copulation ($F_{1,58}$=0.67, p=0.418) (*Table 4B, Table 5B*). There is a higher amount of variation in the model with F2, than the model without F2 (*Table 5A, 5B*). Some of the variation in the model with F2 is attributable to knowing the identity of each chameleon, however, almost all of the variation in the model without F2 is explained by the individual term (*Table 5A, 5B*).

Hoots and rumbles were analyzed separately in females since these calls differ both in duration and frequency (Laslie, 2018). Female hoot frequency differed significantly with both receptivity of the female ($F_{1,421}$=4.1, p=0.043) and SVL ($F_{1,421}$=18.5, p < 0.001) (*Table 6A, Table 7A*). The mean dominant hoot frequency in receptive females (mean=225.3 Hz) was significantly higher in than that of non-receptive females (mean=137.8 Hz). There is a negative relationship between hoot frequency and SVL in receptive individuals, however, size does not have an effect on hoot frequency in non-receptive individuals (*Figure 8*). There was a high amount of variation in the model (*Table 7A),* none of which was contributable to the identitiy of each chameleon. Hoot duration in females was not affected by receptivity or SVL. Female rumble duration was significantly longer ($F_{1,210.99}$=30.4, p<0.001) in trials with non-receptive females (mean=1.633 s) than in trials with receptive females (mean=0.901 s; *Table 6B, Table 7B*). Rumble frequency of females differed with the interaction between receptivity of females and SVL ($F_{1,209}$=7.0, p=0.009; *Table 6C, Table 7C*). Rumble frequency was higher when females were non-receptive (mean=129.6 Hz) than when females were receptive

(mean=92.8 Hz). An interaction plot between female rumble frequency and size indicates a positive relationship between in receptive individuals, and a negative relationship between the two variables in non-receptive individuals (*Figure 9*).

Behavioral context had no effect on hoot duration; however, it did affect hoot frequency ($F_{1,135.4}$=10.9, p=0.001). Dominant frequency of male hoots was higher in courtship trials (mean=133.5 Hz) compared to territorial trials (mean=129.8 Hz) (*Table 8, Table 9*). Rumble frequency and duration, on the other hand, were not affected by behavioral context.

DISCUSSION

Reciprocal calling

The first objective of this study was to determine whether or not there was reciprocal calling between male and female veiled chameleons during courtship and breeding. Calls between chameleons were considered to be reciprocal when the second individual responded to the first individual's call within a 20-second period. Male and female veiled chameleons were found to produce reciprocal calls. However, the amount of trials including reciprocal calls was lower than expected, with only 14 of 37 trials containing reciprocal calling. The majority of trials that showed back-and-forth communication between both individuals had occurred within a 20-second time period, with few trials consisting of inter-call intervals over 20 seconds (see Figure 5).

Previous studies have shown response times between the range of less than 1 second (the second individual called prior to the first individual's call stopping) in cotton-top tamarins (*Saguinus Oedipus*; Kureta, 2000) and 15 seconds in moustached tamarins (*Saguinus mystax*) (Snowdon and Hodun, 1985). Between these two extremes, bottlenose dolphins (*Tursiops truncatus*) were found to vocal match each other with an average of 0.56 seconds (King et al., 2014). Similarly, the white-nosed shrub frog (*Philautus leucorhinus*) was also found to respond to calls within an average of 0.5 seconds (Arak, 1983). While these examples exhibit much shorter response times than those found in *C. calyptratus*, all of these animals (e.g., dolphins, tamarins, and frogs) are social animals that live in groups which likely requires quicker response times to calls because of the competitive nature of group calling. Also, each has more sophisticated hearing which underlines the importance of quicker response times. Chameleons are more solitary

animals with poor hearing (Nečas, 1999; Tolley and Herrel, 2013), which likely hinders their response times. Additionally, two animals communicating with sound are able to produce signals simultaneously while still hearing and comprehending the signals produced by the other animal. While interrupting can still result in effective communication with audible sounds, it is highly unlikely that this is the case for substrate-borne vibrations with long intervals between calls. When communicating with vibrations through a substrate, multiple signals being produced simultaneously would result in vibrations encountering each other through the medium, producing constructive or destructive interference which could modify the original signals. This problem is avoided by using a relatively longer latency period between separate calls.

Call rate

Call rate was only found to differ with the type of trial (i.e., copulation or no copulation) when F2 was included in the model. However, due to the amount of variation in that model that was attributable to random effects compared to that of the model excluding F2, it is unclear if these results were biologically significant (see *Figure 7*). It would be more conservative to conclude that these results do not yield biological significance due to the lack of research on this topic.

Female call characteristics and receptivity

The second objective of this study was to determine whether or not female call characteristics differed with female receptivity. Both female reproductive status and size (SVL) had an effect on many aspects of female *C. calyptratus* calls. Receptive females

produced hoots with higher frequencies than non-receptive females. To date, there has been limited research, if any, on the effects of female chameleon receptivity on female call characterisitics, like duration and dominant frequency. However, the differences in these factors based on receptivity indicate the possibility that female *C. calyptratus* could use biotremors to advertise their reproductive status to potential mates.

Female rumble duration was also affected by receptivity. Non-receptive females produced longer rumbles than receptive ones. Previous studies have shown that longer duration calls will have higher energetic costs than shorter duration calls (Prestwich, 1994; Welch et al., 1998). If this is the case for *C. calyptratus*, one might assume that non-receptive females would not put more energy into calling than receptive ones. Then again, receptive females can conserve energy for reproduction by using shorter rumbles.

Size also affected both female hoot and rumble frequencies (see *Table 6A,6C; Table 7A,7C*). While smaller receptive females produced higher frequency hoots than larger ones, there was no relationship between frequency and size in non-receptive females. Previous studies regarding acoustic allometry have typically shown a negative relationship between body size and call frequency (Bradbury and Vehrencamp, 2011; Martin et al., 2011; Wang et al., 2012; Gingras et al., 2013; Irschick et al., 2015). Wallschläger (1980) found that body size explained 59% of variance in song frequency in an analysis of the songs of 90 European passerine species, more specifically, there was a negative relationship between body size and song frequency. Similarly, multiple studies in frogs have found that as body size increases, call frequency decreases (Wang et al., 2012; Gingras et al., 2013). While this was the case for receptive females, non-receptive females showed no correlation between hoot frequency and size. It appears that

nonreceptive females produce the same call frequency to announce their reproductive status. A consistent call frequency in this regard would likely be selected for to ensure the signal is understood by males.

Size also affected rumble frequency in both non-receptive and receptive females. For receptive females, smaller individuals produced lower frequency rumbles, while larger individuals produced lower frequency rumbles in non-receptive females. In this case, the relationship between size and rumble frequency in receptive females is the opposite of what is shown in female hoot frequency and the previous studies discussed (Wallschläger, 1980; Wang et al., 2012; Gingras et al., 2013). This inconsistency could also be due to low sample sizes, as well as the lack of a broad range of sizes. Three of the five females were 155mm or larger, one individual was 113mm and the other was 131mm. Had there been an even number of "small", "medium", and "large" individuals, the results might have been more similar to those typically found, with larger individuals producing lower frequency calls. All this withstanding, lower frequency calls by smaller individuals could happen as a result as the same frequency modulation mechanism described above and could have implications for signal transduction.

Male call characteristics and behavioral context

In the male-only dataset, the only variable distinguishing male courtship calls from male territorial calls was hoot frequency. Courting males produced hoots with higher frequencies than did males demonstrating territorial behavior. This could potentially illustrate how males can advertise their availability to females while also letting other males know they are courting a female and to avoid the area. While it was

determined that hoot frequency differed based on behavioral context, nothing else differed between contexts. Ideally, another fixed effect could have been added to the model had both territoriality and courtship contexts been recorded for this study; however, because only territorial data was used from a previous dataset (Laslie, 2018), and only courtship data was recorded for this dataset, the model had less terms to take into account variability and most likely lacked power for this reason.

In courtship contexts, females often select males that produce lower frequency calls, which is typically an indicator of body size/condition (Márquez and Bosch, 1997; Howard and Young, 1998; Lodé and Le Jacques, 2003; Halfwerk et al., 2011). Females in two species of toad, *Bufo americanus* and *Alytes obstetricans,* have been shown to be more attracted to males that produced lower frequency calls (Márquez and Bosch, 1997; Lodé and Le Jacques, 2003). Similarly, Lesbarrères et al. (2008) found a negative relationship between male reproductive success (number and size of clutches) and fundamental frequency of calls in the agile frog, *Rana dalmatina*. Conversely, Halfwerk et al. (2011) demonstrate that while female great tits (*Parus major*) prefer males producing lower-frequency songs, there is an advantage for higher-frequency songs in noisy environments. They found that low frequency songs showed reduced effectiveness in soliciting female responses in noisy, urban habitats (Halfwerk et al., 2011). In this study, any background noise during trials was greatly reduced with a sound-proof room. However, veiled chameleons are known to inhabit anthropogenically disturbed habitats, such as tree alleys along busy roadways and trees in villages (Nečas, 1999).

General discussion

Overall, these results, specifically, reciprocal calling, support the notion that substrate-borne vibrational communication is utilized by *C. calyptratus* in conspecific interactions (Barnett et al., 1999; Laslie, 2018; Tegge, 2018). Barnett et al. (1999) recorded mean call frequencies between 50-149 Hz in *C. calyptratus* when males were presented with females and were also able to elicit alarm calls with a mean frequency of 152 Hz. Similarly, Tegge (2018) found mean alarm call frequencies of 133 and 154 Hz in male and female veiled chameleons, respectively. In this study, mean call frequencies in the courtship trials ranged from 75 Hz to 227 Hz.

A previous study showed that *C. calyptratus* can detect low frequency (below 300 Hz) vibrations on a dowel (Laslie, 2018). This suggests that they should also be able to detect the low frequency biotremors recorded in the current study. It is currently unclear which sensory modality chameleons use to detect these vibrations: the auditory system in which substrate-borne vibrations can be transmitted to the inner ear through bone conduction, the somatosensory system via mechanoreceptors in the skin, or both. Wever (1968, 1969) reported that two other species of *Chamaeleo*, *C. senegalensis* and *C. quilensis*, displayed the greatest auditory sensitivity in the range of 200-600 Hz with moderate sensitivity at 100 Hz. This suggests that the auditory system could be used to detect these biotremors. Huskey et al. (2020) also found that *C. senegalensis* was one of the species known to possess a gular pouch and it is highly likely that *C. quilensis* does as well. Based on the correlation between these findings, it is reasonable to assume that the species possessing a gular pouch are able to amplify the biotremors they produce in order to allow surrounding conspecifics to receive these signals more effectively.

Veiled chameleons are known to use biotremors to communicate with conspecifics in the contexts of courtship and territoriality (Barnett et al., 1999; Laslie, 2018), as well as alarm calls with heterospecifics (Barnett et al., 1999; Laslie, 2018; Tegge, 2018). *C. calyptratus* likely evolved the use of substrate-borne vibrations in communication for a number of reasons, one of which being the environmental pressures of an arboreal habitat. Adult *C. calyptratus* are typically found on trees and shrubs (Nečas, 1999). As demonstrated by Barnett et al. (1999), a shrub branch is an ideal substrate through which these vibrations can be transmitted from one individual to another. Another pressure that could be responsible for the adaptation of producing biotremors to communicate would be the poor sense of hearing found in chameleons. The ear in all chameleons is greatly reduced; there is no external ear opening or tympanic membrane, and the round window is either absent or greatly reduced (Wever, 1968; Wever, 1969; Tolley and Herrel, 2013). Substrate-borne vibrations might provide a means of communication in spite of a chameleon's elementary hearing

Within a courtship context, female chameleons are typically able to advertise their receptivity by coloration or visual behaviors (Kelso and Verrel, 2002; Tolley and Herrel, 2013). Because characteristics of female hoots and rumbles were found to differ by receptivity of the female, it is reasonable to postulate that females can communicate their receptivity via biotremors while out of the range of sight for courting males. Similarly, males could also be doing the same when advertising their availability to receptive females where call frequency, duration, or amplitude could provide sexually selective information. Males could also be advertising their physical prowess to rival suitors in a show of dominance. However, since only hoot frequency was higher in courting males

than territorial ones, further investigation on male biotremors based on behavioral context would be necessary in drawing that conclusion.

In addition to one mode of sensory communication being used when another is less than ideal, there is also the possibility of both modes of communication (visual and vibratory) being utilized simultaneously. Multimodal signaling is found throughout the animal kingdom (Uetz et al., 2009; Higham and Hebets, 2013; Preininger et al., 2013). For example, male wolf spiders (Lycosidae) are known to incorporate visual and vibratory signals in their courtship displays (Uetz et al., 2009). In Uetz et al.'s study (2009), they found that female wolf spiders responded to multimodal signals from males much quicker than the visual and vibratory cues alone. Similarly, the small torrent frog (*Micrixalus saxicola*) exhibits multimodal signaling with acoustic and visual signals in male-male territorial context (Preininger et al., 2013). Preininger et al. (2013) found that multimodal signals significantly increased the frequency of responses compared to the unimodal signals. Both of these examples support the notion that the redundancy of multimodal signals actually enhances the ability of the receiver to obtain the information being sent (Uetz et al., 2009; Preininger et al., 2013). While female veiled chameleons are able to display their reproductive status via coloration and visable behaviors, adding vibrations could be adding emphasis to what they are already communicating, and still could provide this information to any individual out of eyesight.

Conclusions

The present study demonstrates the ability of female veiled chameleons to communicate their receptivity through the use of substrate-borne vibrations. The

evidence presented here, as well as the anatomical ability for veiled chameleons to produce biotremors (Tegge, 2018; Huskey et al., 2020), and previous biotremor data collected (Barnett et al., 1999; Laslie, 2018, Tegge, 2018) supports the concept that *C. calyptratus* is able to utilize biotremors to communicate in different contexts. However, more investigation is necessary in order to compile a larger repertoire of chameleon biotremors and determine how these vibrations differ by context, sex, receptivity, and other potentially important factors.

REFERENCES

Annis, J. M. 1995. Veiled chameleon (*Chamaeleo calyptratus*): natural history, captive management, and breeding, p. 77-99. *In*: Care and Breeding of Panther, Jackson's, Veiled, and Parson's Chameleons. P. deVosjoli, and G. Ferguson (eds). Advanced Vivarium Systems, Inc, Santee, California.

Arak, A. 1983. Vocal interactions, call matching and territoriality in a Sri Lankan treefrog, *Philautus leucorhinus* (Rhacophoridae). Animal Behavior 31: 292-302.

Barnett, K. E., R. B. Cocroft, and L. J. Fleishman. 1999. Possible communication by substrate vibration in a chameleon. Copeia 1999: 225-228.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. Fitting linear mixed-effects models using lme4. Journal of Statistical Software 67(1): 1-48.

Boka, K. 2012. What's that hooting sound? A survey on novel sound producing mechanisms in chameleons. Unpubl. Honor's thesis, Western Kentucky University, Bowling Green, Kentucky.

Bradbury, J. L., and S. L. Vehrencamp. 2011. Principles of animal communication. Second edition. Sinauer Associates, Sunderland, Massachusetts.

Christensen, C. B., J. Christensen-Dalsgaard, B. Christian, and P. T. Madsen. 2012. Hearing with an atympanic ear: Good vibration and poor sound-pressure detection in the royal python, *Python regius*. Journal of Experimental Biology 215: 331-342.

Cocroft, R. B., and R. L. Rodríguez. 2005. The behavioral ecology of insect vibrational communication. Bioscience 55: 324-334.

Cocroft, R. B., H. J. Shugart, K. T. Konrad, and K. Tibbs. 2006. Variation in plant

substrates and its consequences for insect vibrational communication. Ethology 112: 779-789.

Dawkins, R., and J. R. Krebs. 1978. Animal signals: information or manipulation? p. 282-309. *In* Behavioral ecology, an evolutionary approach. First edition. J. R. Krebs, and N. B. Davies (eds). Sinauer Associates, Sunderland, Massachusetts.

Diaz, Jr., R. E., C. V. Anderson, D. P. Baumann, R. Kupronis, D. Jewell, C. Piraquive, J. Kupronis, K. Winter, F. Bertocchini, and P. A. Trainor. 2015. The veiled chameleon: A model for studying reptile body plan development and evolution. Cold Spring Harbor Protocols 2015(10): 889-894.

Endler, J. A. 1993. Some general comments on the evolution and design of animal communication systems. Philosophical Transcripts of the Royal Society of London (B) 340(1292): 215-225.

Gary, N. E. 1962. Chemical mating attractants in the queen honey bee. Science 136: 773-774.

Germershausen, V. G. 1913. Anatomische untersuchungen über den kelkopf der Chamaeleonen. Sitzungsberichte der Gesellschaft Naturforschender Freunde zu Berlin 191: 462-535.

Gingras, B., M. Boeckle, C. T. Herbst, and W. T. Fitch. 2013. Call acoustics reflect body size across four clades of anurans. Journal of Zoology 289: 143-150.

Halfwerk, W., S. Bot, J. Buikx, M. van der Velde, J. Komdeur, C. ten Cate and H. Slabbekoorn. 2011. Low-frequency songs lose their potency in noisy urban conditions. PNAS 108(35): 14549-14554.

Harkness, L. 1977. Chameleons use accommodation cues to judge distance. Nature 267(5609): 346-349.

Hartline, P. H. 1971. Physiological basis for detection of sound and vibration in snakes. Journal of Experimental Biology 54: 349-371.

Herrel, A., J. J. Meyers, P. Aerts, and K. C. Nishikawa. 2000. The mechanics of prey prehension in chameleons. Journal of Experimental Biology 203: 3255-3263.

Higham, J. P., and E. A. Hebets. 2013. An introduction to multimodal communication. Behavioral Ecology and Sociobiology 67: 1381-1388.

Hill, P. S. M. 2009. How do animals use substrate-borne vibrations as an information source? Naturwissenschaften 96: 1355-1371.

Hill, P. S. M., and A. Wessel. 2016. Biotremology. Current Biology 26: 187-191.

Hödl, W., and A. Amézquita. 2001. Visual signaling in anuran amphibians, p. 121-141. *In* Anuran communication. M. J. Ryan (ed). Smithsonian Institution Press, Washington, D.C.

Howard, R. D. and J. R. Young. 1998. Individual variation in male vocal traits and female mating preferencs in *Bufo americanus*. Animal Behavior 55(5): 1165-1179.

Huskey, S. 2017. The skeleton revealed: An illustrated tour of the vertebrates. John Hopkins University Press, Baltimore, Maryland.

Huskey, S., S. M. Tegge, C. V. Anderson, M. E. Smith, and K. Barnett. 2020. Gular pouch diversity in the Chamaeleonidae. The Anatomical Record 2020: 1-14.

Irschick, D. J., M. Briffa, and J. Podos. 2015. Animal signaling and function: an integrative approach. John Wiley & Sons, Inc., Hoboken, New Jersey.

Kelso, E. C., and P. A. Verrell. 2002. Do male veiled chameleons, *Chamaeleo calyptratus*, adjust their courtship displays in response to female reproductive status? Ethology 108: 495-512.

King, S. L., H. E. Harley, and V. M. Janik. 2014. The role of signature whistle matching in bottlenose dolphins, *Tursiops truncatus*. Animal Behavior 96: 79-86.

Krysko, K. L., K. M. Enge, and F. W. King. 2004. The veiled chameleon, *Chamaeleo calyptratus*: A new exotic lizard species in Florida. Florida Scientist 67: 249-253.

Kureta, Y. 2000. Vocal exchanges between familiar cotton-top tamarins during social isolation. Japanese Journal of Animal Psychology 50(1): 95-102.

Kuznetsova A., P. B. Brockhoff, and R. H. B. Christensen. 2017. lmerTest Package: tests in linear mixed effect models. Journal of Statistical Software 82: 1-26.

Laslie, K. C. 2018. Investigations of biotremors in the veiled chameleon (*Chamaeleo calyptratus*). Unpubl. Master's thesis, Western Kentucky University, Bowling Green, Kentucky.

Laumann, R. A., M. C. B. Moraes, A. Čokl, and M. Borges. 2007. Eavesdropping on sexual vibratory signals of stink bugs (Hemiptera: Penraromidae) by the egg parasitoid *Telenomus podisi*. Animal Behavior 73: 637-649.

Lesbarrères, D., J. Merilä and T. Lodé. 2008. Male breeding success is predicted by call frequency in a territorial species, the agile frog (*Rana dalmatina*). Canadian Jounal of Zoology 86: 1273-1279.

Lodé, T. and D. Le Jacques. 2003. Influence of advertisement calls on reproductive success in the male midwife toad *Alytes obstetricans*. Behavior 140(7): 885-898.

Markl, H. 1983. Vibrational communication, p. 332-353. *In* Neuroethology and Behavioral Physiology. F. Huber and H. Markl (eds). Springer-Verlag, Berlin, Germany.

Márquez, R. and J. Bosch. 1997. Male advertisement call and female preference in sympatric and allopatric midwife toads. Animal Behavior 54(6): 1333-1345.

Martin, J. P., S. M. Doucet, R. C. Knox, and D. J. Mennill. 2011. Body size correlates negatively with the frequency of distress calls and songs of Neotropical birds. Journal of Field Ornithology 82(3): 259-268.

Mates, J. W. B. 1978. Eye movements of African chameleons: spontaneous saccade timing. Science 199: 1087-1088.

Morton, E. S., and M. D. Shalter. 1977. Vocal response to predators in pair-bonded Carolina wrens. The Condor 79: 222-227.

Nečas, P. 1999. Chameleons: Nature's hidden jewels. Second edition. L. W. Porras (ed). Krieger Publishing Company, Malabar, Florida.

Ott, M., and F. Schaeffel. 1995. A negatively powered lens in the chameleon. Nature 373: 692-694.

Otte, D. 1974. Effects and functions in the evolution of signaling systems. Annual Review of Ecology, Evolution, and Systematics 1974: 385-417.

Pimm, R. H., C. Dutton, S. O'Handley, and G. F. Mastromonaco. 2014. Assessment of the reproductive status of female veiled chameleons (*Chamaeleo calyptratus*) using hormonal, behavioural, and physical traits. Zoo Biology 9999: 1-13.

Preininger, D., M. Boeckle, A. Freudmann, I. Starnberger, M. Sztatecsny, and W. Hödl. 2013. Multimodal signaling in the small torrent frog (*Micrixalus saxicola*)

in a complex acoustic environment. Behavioral Ecology and Sociobiology 67: 1449-1456.

Prestwich, K. N. 1994. The energetics of acoustic signaling in anurans and insects. American Zoology 34: 625-643.

Rand, A. S., and M. J. Ryan. 1981. The adaptive significance of a complex vocal repertoire in a neotropical frog. Zeitschrift für Tierpsychologie 57: 209-214.

Römer, H., A. Lang, and M. Hartbauer. 2010. The signaler's dilemma: a cost-benefit analysis of public and private communication. PLoS One 5(10): e13325.

Ryan, M. J. 1980. Female mate choice in a neotropical frog. Science 209: 523-525.

Sándor, P. S., M. A. Frens, and V. Henn. 2001. Chameleon eye position obeys Listing's law. Vision Research 41: 2245-2251.

Seyfarth, R. M., and D. L. Cheney. 2003. Signalers and receivers in animal communication. Annual Review of Psychology 54: 145-173.

Snowdon, C. T., and A. Hodun. 1985. Troop-specific responses to long calls of isolated tamarins (*Saquinus mystax*). American Journal of Primatology 8: 205-213.

Stuart-Fox, D., and A. Moussalli. 2008. Selection for social signaling drives the evolution of chameleon colour change. PLoS Biology 6(1): 0022-0029.

Tegge, S. 2018. The mechanism of biotremor production in the veiled chameleon (*Chamaeleo calyptratus*). Unpubl. Master's thesis, Western Kentucky University, Bowling Green, Kentucky.

Tolley, K. A., and A. Herrel. 2013. The biology of chameleons. First edition. K. A. Tolley and A. Herrel (eds). University of California Press, Berkeley, California.

Tornier, G. 1905. Bau und betätigung der kopflappen und halsluftsäcke bei chamäleon.

Zoologische Jahrbuecher Abteilung fuer Anatomie und Ontogenie der Tiere 21: 1-40.

Uetz, G. W., J. A. Roberts, and P. W. Taylor. 2009. Multimodal communication and mate choice in wolf spiders: female response to multimodal versus unimodal signals. Animal Behavior 78: 299-305.

Virant-Doberlet, M., and A. Cokl. 2004. Vibrational communication in insects. Neotropical Entomology 33(2): 121-134.

Wall, G. L. 1942. The vertebrate eye and its adaptive radiation. Hafner Publishing Company, New York, New York.

Wallschläger, D. 1980. Correlation of song frequency and body weight in passerine birds. Experientia 36: 412.

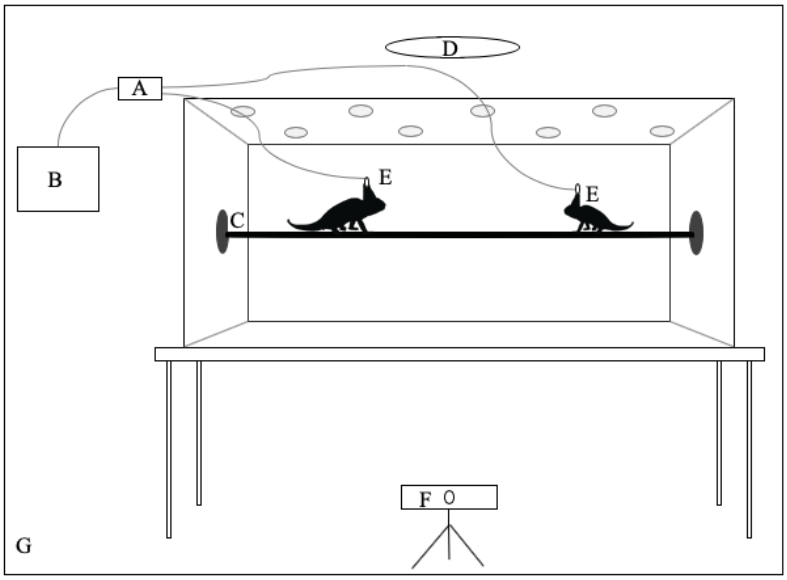
Wang, J., **J. Cui, H. Shi, S. E. Brauth, and Y. Tang.** 2012. Effects of body size and environmental factors on the acoustics structure and temporal rhythm of calls in *Rhacophorus dennysi*. Asian Herpetological Research 3(3): 205-212.

Welch, A. M., R. D. Semlitsch and H. C. Gerhardt. 1998. Call duration as an indicator of genetic quality in male gray tree frogs. Science 280: 1928-1930.

Wever, E. G. 1968. The ear of the chameleon: *Chamaeleo senegalensis* and *Chamaeleo quilensis.* Journal of Experimental Biology 168: 423-436.

Table 1: Chameleons used in experiment. Listed are the individual chameleons, with their respective snout-vent lengths (SVL) (mm), and mass (g).

Individual	SVL (mm)	Mass (g)
F1	131	71.83
F2	113	61.07
F3	161	108.10
F4	155	87.97
F5	170	99.84
M1	190	192.90
M2	182	136.33
M3	128	66.44
M4	205	133.30
M5	164	79.30



A. National Instruments C Series Sound and Vibration Input Module
B. Video DAQ Acquisition and Video DAQ Analysis software (courtesy of Doug Harper, WKU)
C. Wooden dowel (101.6 x 1.5 cm)
D. LED light
E. PCB accelerometer (394C06)
F. Logitech C922 Pro Webcam
G. WhisperRoom, Inc. Sound Isolation Enclosure (SE 2000)

Figure 1: Schematic of experimental setup.

Figure 2: A male *C. calyptratus* with an accelerometer attached to the casque with petro wax. Adapted from Tegge (2018).

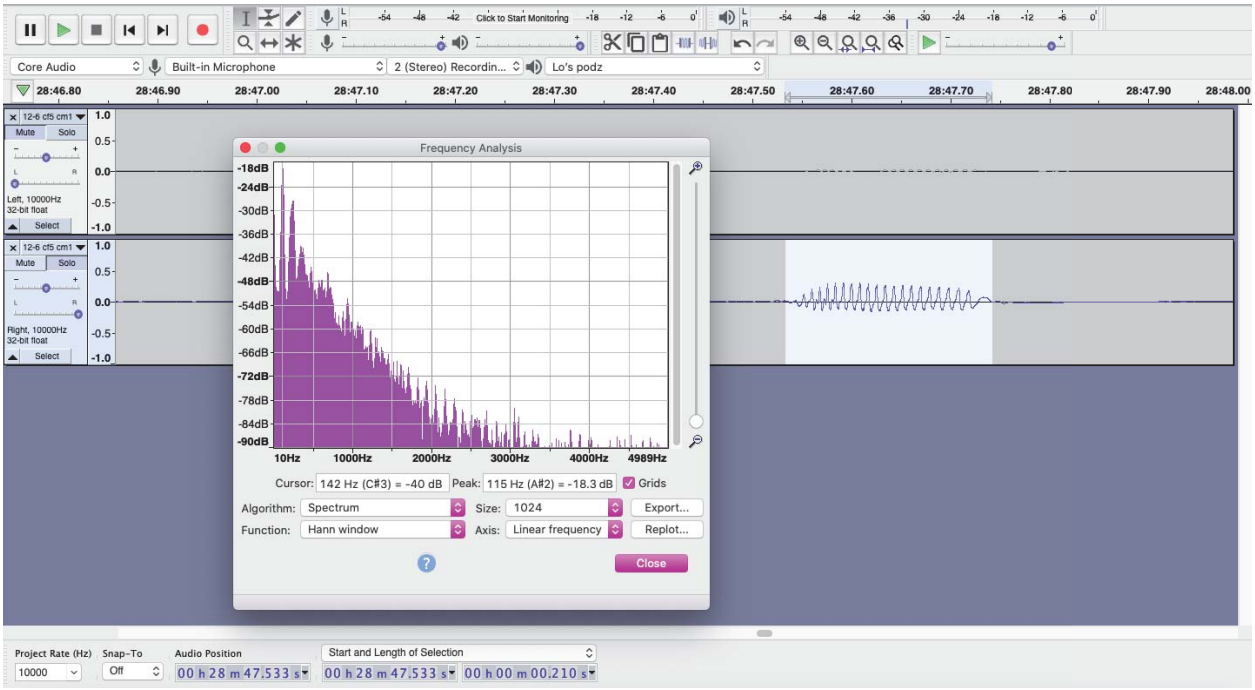


Figure 3: Screenshot of Audacity analysis software (version 2.3.2). A hoot produced by an adult male *C. calyptratus* is highlighted and analyzed. The analysis shows the hoot is 0.210 s in duration with a dominant frequency of 115 Hz.

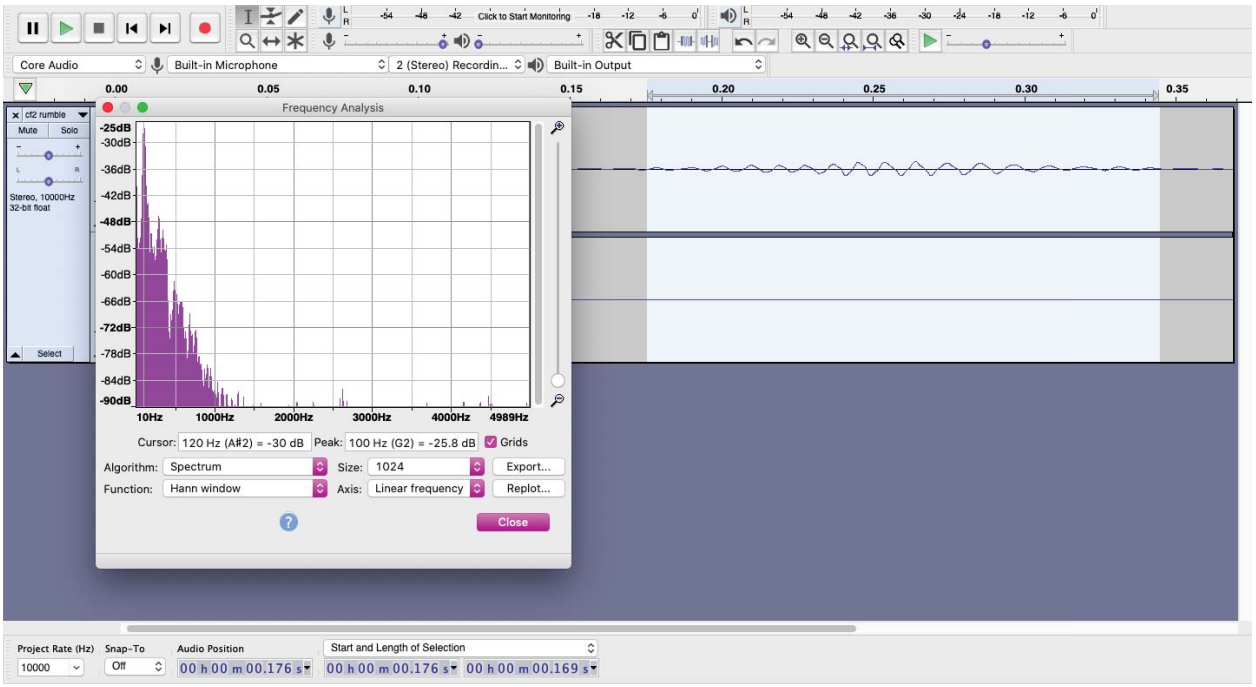


Figure 4: Screenshot of Audacity analysis software (version 2.3.2). A rumble produced by an adult female *C. calyptratus* is highlighted and analyzed. The analysis shows the rumble is 0.169 s in duration with a dominant frequency of 100 Hz.

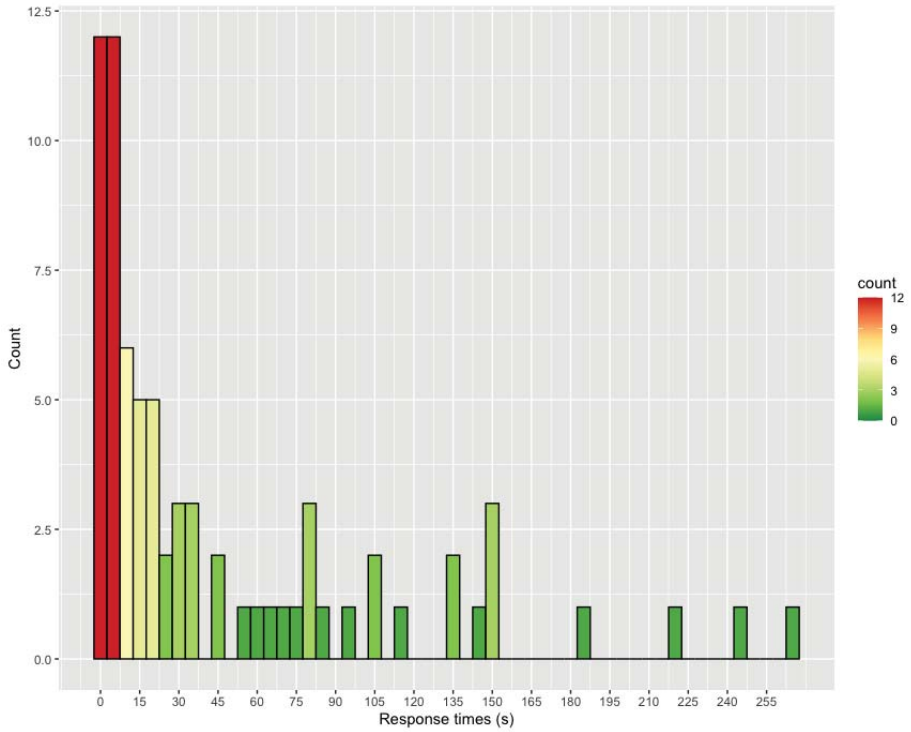


Figure 5: Histogram of inter-call intervals (response times) from all trials with both male and female calls present.

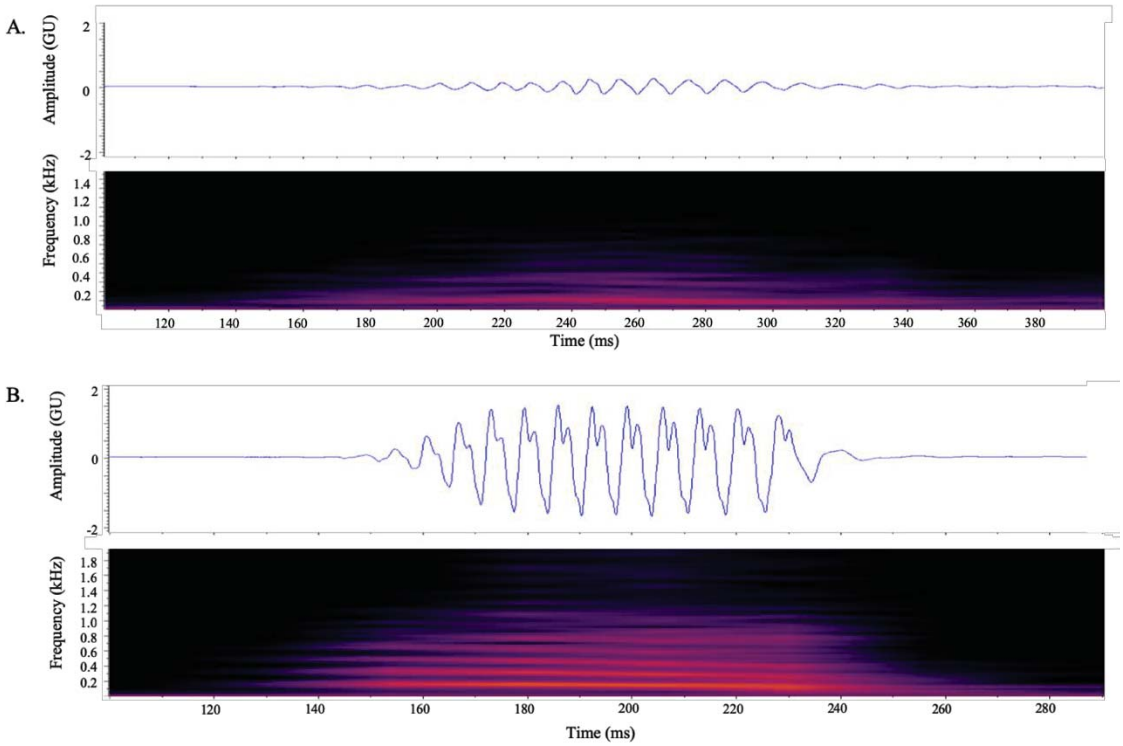


Figure 6: One rumble (A) and one hoot (B) analyzed from a single female *C. calyptratus* (CF2) in a single male-female courtship trial.

Table 2: Mean duration (s) and mean dominant frequency (Hz) of hoots and rumbles from each individual. *M3 was removed as he never produced calls.

Individual	Hoots		Rumbles	
	Mean Duration (s)	Mean Dominant Frequency (Hz)	Mean Duration (s)	Mean Dominant Frequency (Hz)
F1	0.131 (±0.0004)	155.5 (±28.6)	1.614 (±3.1)	97.5 (±155.9)
F2	0.137 (±0.0001)	227.2 (±278.4)	0.823 (±0.2)	94 (±207.3)
F3			0.521 (±0.02)	93.4 (±135.2)
F4	0.159 (±0.012)	147.2 (±266.0)	1.145 (±0.6)	127.7 (±271.3)
F5	0.126 (±0.001)	119.8 (±323.3)	3.775 (±2.5)	164.2 (±2094.2)
M1	0.259 (±0.004)	129.1 (±168.3)	0.468 (±0.04)	88.5 (±171.1)
M2	0.126 (±0.002)	137.3 (±91.9)	0.678 (±0.2)	75.2 (±438.4)
M4	0.155 (±0.001)	131.3 (±18.8)	0.571 (±0.1)	86.2 (±50.4)
M5			2.878 (±3.1)	125.1 (±554.2)

Table 3: Trials with reciprocal calling present.

Trial	Chameleons (female, male)	Female receptivity	Number of reciprocal calls	Average time lag between reciprocal calls (sec)	Total number of calls in trial	Percentage of reciprocal calls	Did copulation/"mounting" occur?
2	F2, M1	R	4	4.89	112	3.57	Y
4	F1, M1	NR	2	0.27	14	28.57	N
6	F2, M1	R	3	2.12	91	3.30	Y
7	F2, M2	R	2	2.82	120	1.67	Y
10	F2, M1	R	1	6.77	13	7.69	Y
12	F2, M2	R	1	18.40	84	1.22	Y
22	F3, M5	NR	2	14.79	13	7.69	N
24	F2, M5	NR	5	11.96	15	26.67	N
30	F5, M1	R	3	5.91	20	15.00	Y
32	F5, M4	R	2	6.31	17	11.77	N
34	F2, M5	NR	6	3.36	17	35.29	N
37	F5, M5	R	4	9.22	14	21.43	N
40	F5, M2	R	1	12,86	31	3.23	Y
48	F5, M1	R	2	8.12	27	7.41	Y

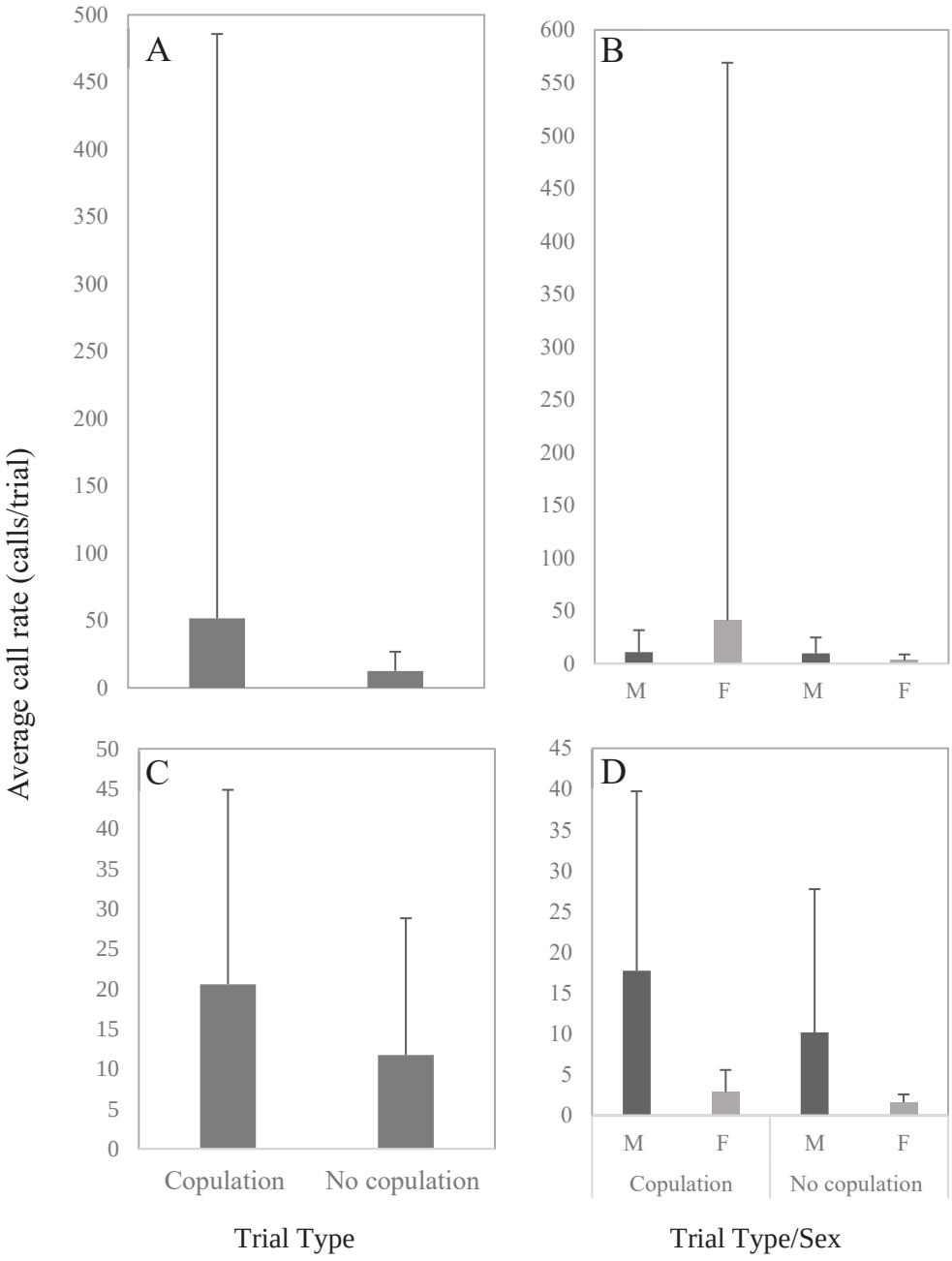


Figure 7: Average call rates (calls/trials) in trials with and without copulation. Plots A and C represent the pooled dataset (male and females), with A containing trials with F2 and C containing all other trials without F2. Plots B and D contain call rate data separated by sex, with plot B containing trials with F2, and D containing all other trials without F2. SE of each mean are denoted by vertical error bars.

Table 4: Linear mixed-effect regression model comparison of factors affecting call rate with F2 (A) and without F2 (B). Models were constructed with the fixed effect being "Copulation" (whether or not copulation occurred). The best model in each set is denoted by an asterisk beside the AIC value.

			Factors affecting call rate					
A	Model	df	AIC	ΔAIC	LogLik	X^2	X^2 df	P
1. Null		3	666.5	4.4	-330.2			
2. Copulation		4	*662.1	0	-327.0	6.41	1	0.011

			Factors affecting call rate					
B	Model	df	AIC	ΔAIC	LogLik	X^2	X^2 df	P
1. Null		3	*416.5	0	-205.3			
2. Copulation		4	417.9	1.4	-205.0	0.61	1	0.435

Table 5: Parameter estimates for ANOVAs on differences call rate with F2 (A) and without F2 (B). Models were constructed as mentioned in *Table 4*.

A.

Random Effects	Variance	SD		
Individual	196.8	14.0		
Residual	334.3	18.3		
Fixed Effects	Estimate	SE	T	P-value
Intercept	18.11	5.797		
Copulation	-12.29	4.830	-2.545	0.013

B.

Random Effects	Variance	SD		
Individual	25.0	5.0		
Residual	35.2	5.9		
Fixed Effects	Estimate	SE	T	P-value
Intercept	4.5	2.168		
Copulation	1.4	1.755	0.816	0.418

Table 6: Linear mixed-effect regression model comparison of factors affecting female hoot frequency (A), rumble duration (B), and rumble frequency (C). Models were constructed with fixed effects being "Receptivity" (receptiveness of female), "Receptivity + SVL" (additive model with SVL as a covariate, and "Receptivity*SVL" (interaction between receptivity of female and SVL). The best model in each set is denoted by an asterisk beside the AIC value.

Factors affecting female hoot frequency

A Model	df	AIC	ΔAIC	LogLik	X^2	X^2 df	P
1. Null	3	4865.7	10.5	-2429.9			
2. Receptivity	4	4862.9	7.7	-2427.5	4.785	1	0.029
3. Receptivity + SVL	5	*4855.2	0	-2422.6	9.703	1	0.002
4. Receptivity*SVL	6	4855.2	0	-2421.6	2.020	1	0.155

Factors affecting female rumble duration

B Model	df	AIC	ΔAIC	LogLik	X^2	X^2 df	P
1. Null	3	681.6	25.4	-337.8			
2. Receptivity	4	*656.2	0	-324.1	27.419	1	<0.001
3. Receptivity + SVL	5	656.5	0.3	-323.3	1.656	1	0.198

Factors affecting female rumble frequency

C Model	df	AIC	ΔAIC	LogLik	X^2	X^2 df	P
1. Null	3	419.4	16.0	-206.7			
2. Receptivity	4	411.0	7.6	-201.5	10.394	1	0.001
3. Receptivity + SVL	5	408.4	5.01	-199.2	4.607	1	0.032
4. Receptivity*SVL	6	*403.4	0	-195.7	7.011	1	0.008

Table 7: Parameter estimates for ANOVAs on differences in female hoot frequency (A), female rumble duration (B), and female rumble frequency (C). Models were constructed as mentioned in *Table 4*.

A.

Random Effects	Variance	SD		
Individual	0	0		
Residual	5414	73.58		
Fixed Effects	Estimate	SE	T	P-value
Intercept	364.6	58.8		
Receptivity	55.5	27.3	2.034	0.043
SVL	-1.7	0.4	-4.303	< 0.001

B.

Random Effects	Variance	SD		
Individual	0.981	0.991		
Residual	1.168	1.081		
Fixed Effects	Estimate	SE	T	P-value
Intercept	0.275	0.491		
Receptivity	-1.084	0.197	-5.510	< 0.001

C.

Random Effects	Variance	SD		
Individual	3.01**e**-16	1.74**e**-8		
Residual	3.75**e**-1	6.12**e**-1		
Fixed Effects	Estimate	SE	T	P-value
Intercept	5.037	0.601		
Receptivity	-2.071	0.679	-3.052	0.003
SVL	-0.003	0.005	-0.581	0.562
Receptivity*SVL	0.014	0.005	2.645	0.009

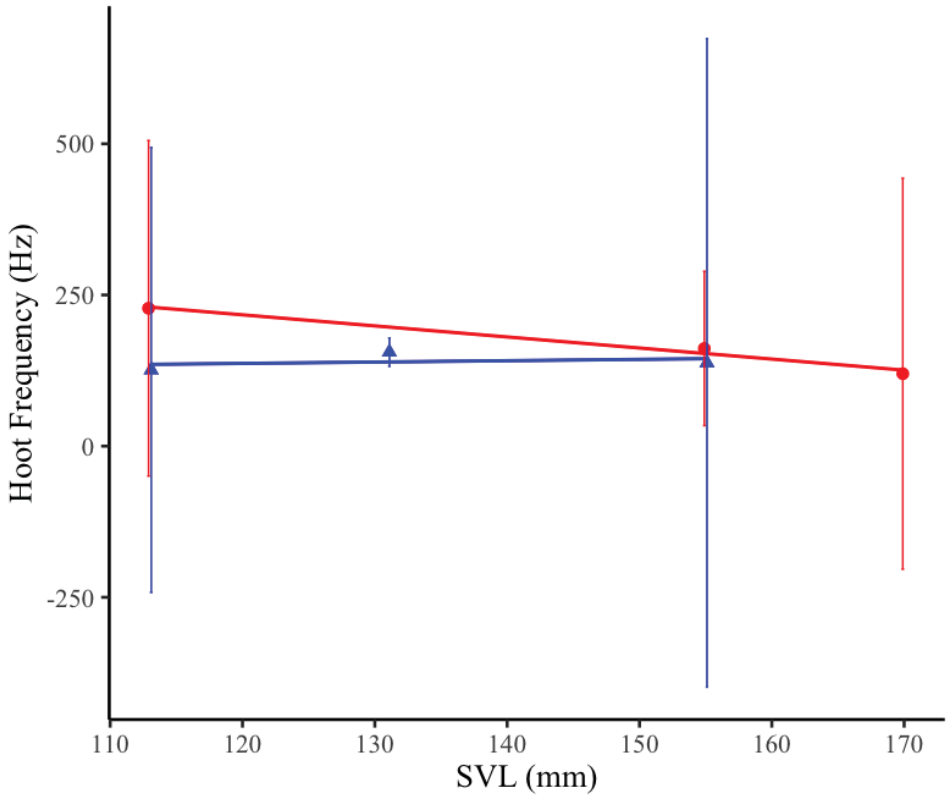


Figure 8: Interaction plot of female mean (± S.E.) dominant hoot frequency (Hz) by SVL (mm), depicting the interactive model shown in *Table 4A* and *Table 5A.* Red circles denote receptive individuals, blue triangles denote non-receptive individuals.

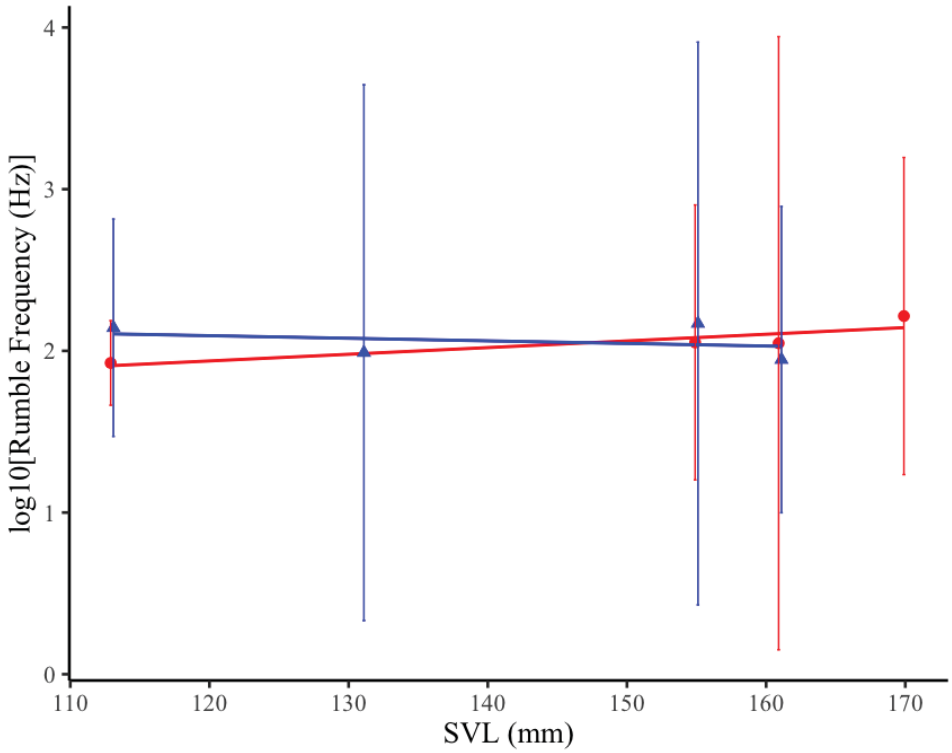


Figure 9: Interaction plot of female mean (± S.E.) dominant rumble frequency (Hz) by SVL (mm), depicting the interactive model shown in *Table 4C* and *Table 5C.* Red circles denote receptive individuals, blue triangles denote non-receptive individuals.

Table 8: Linear mixed-effect regression model comparison of factors affecting male hoot frequency. Models were constructed with fixed effects being "Context" (behavioral context of male – courtship/territoriality). The best model is denoted by an asterisk beside the AIC value

		Factors affecting male hoot frequency					
Model	df	AIC	ΔAIC	LogLik	X^2	X^2 df	P
1. Null	3	3320.9	5.9	-1657.4			
2. Context	4	*3315.0	0	-1653.5	7.820	1	0.005

Table 9: Parameter estimates for ANOVAs on differences in male hoot frequency. Models were constructed as mentioned in *Table 5*.

Random Effects	Variance	SD		
Individual	207.3	14.4		
Residual	1012.9	31.8		
Fixed Effects	Estimate	SE	T	P-value
Intercept	139.4	7.777		
Context	-20.5	6.203	-3.301	0.001